

LVII. FURTHER EXPERIMENTS ON CANCER-PRODUCING SUBSTANCES.

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THE carcinogenic factor in gas-works tar is known to be present in the higher-boiling fractions. A list has been compiled [Kennaway, 1924, 1] of the compounds known to be present in tar with boiling points above 270°, which temperature appeared to indicate very roughly the boundary between the lower-boiling non-carcinogenic, and higher-boiling fractions. The list is reproduced below (Table I) together with the results obtained in this laboratory and others [Bloch, 1922; Twort and Fulton, 1930] by application of some of these compounds to mice.

Table I. *Substances in tar which boil above 270°.*

			Cook, Hieger and Kennaway	Bloch [1922]	Twort and Fulton [1930]
Acenaphthene	-	-	-
Fluorene	-	-	-
Fluoranthene	-	.
Anthracene	-	-	-
Dihydroanthracene	-
Hexahydroanthracene
β -Methylanthracene	-	-	-
Phenanthrene	-	-	-
Retene	-	-	-
Pyrene	under test	-	nil 35 weeks
Chrysene	-	-	+
Picene	-	.	-
Truxene	-	.	-
Naphthacene	-	.	.
Naphthanthracene	under test	.	.
Chrysogene
Benzerythrene	1 papilloma
Crackene
Diphenylene oxide	under test	.	.
β -Naphthofurane
α - and β -Naphthol
α -Dimethylquinoline
Carbazole	-	-	.
Phenyl- <i>p</i> -naphthylcarbazole
Acridine	-	.	.
Diphenylene sulphide

+ and - indicate positive and negative results of application to mice.

The table shows discordant results with chrysene. Chrysene (at first 1 %, then 0.33 % suspension of Kahlbaum's preparation in "90 % benzol" at

40°) was applied here to 100 mice for 11 months with negative results [Kennaway, 1924, 1], but only 5 of these were alive after 6½ months. Twort and Fulton [1930] report 5 cancers and 10 warts in mice painted with chrysene (suspension in oleic acid or liquid paraffin) either alone or with other compounds; apparently they used several hundreds of mice, but they do not state the exact number exposed to chrysene. From the yield of tumours they conclude that the carcinogenic potency of chrysene, in such suspensions, must be very low. The negative results with some of the above substances are not very satisfactory, owing to their very low solubility (*e.g.* naphthacene, truxene, picene). Naphthacene, with dihydronaphthacene, was prepared by Hieger by the method of Gabriel and Leupold [1898]; naphthanthracene, with dihydronaphthanthracene, was prepared by Cook by the method of Clar and John [1929]; in both cases the hydrocarbon and the partially reduced compound were applied together.

SYNTHETIC CARCINOGENIC MATERIALS.

I. *Tars.*

Various attempts have been made to obtain if possible carcinogenic material of a nature simpler than coal tar. Isoprene, acetylene, human skin, yeast, and cholesterol were heated between 700° and 920°, and cancer-producing mixtures, some of them very active, were obtained from each one of these [Kennaway, 1924, 2; 1925; Kennaway and Sampson, 1928]. Human voluntary muscle also, heated to 920° is very effectual (4 papillomata and 12 cancers from 100 mice in 243 days). By these methods extremely active tars have been prepared by Twort and Fulton [1930] from turpentine and from pinene.

II. *Products of the action of aluminium chloride upon tetralin.*

It was thought desirable to seek for carcinogenic compounds formed at lower temperatures. Accordingly the high-boiling mixture of compounds [Schroeter, 1920] obtained by the action of AlCl_3 upon tetrahydronaphthalene (tetralin) was examined; for the sake of brevity this mixture is spoken of here as "Schroeter." (1) 220 g. tetralin were left at 37° for 18 hours with 7.5 % by weight of AlCl_3 , and then decomposed with water in the usual way. The oil was distilled over the free flame to remove tetralin (B.P. 204°) until the temperature in the neck was 270°; the clear viscid reddish-yellow residue was painted at first undiluted, later diluted with 2 vols. xylene, upon 30 mice. All were dead in 148 days, but one papilloma was produced, and the same material produced papillomata upon the ears of 2 rabbits. (2) A repetition of the experiment with two fresh batches of material prepared in the same way gave 11 tumours (8 cancers, 3 papillomata) in 20 mice in 256 days. (3) If a larger proportion of AlCl_3 (36 %) was allowed to act for a longer time (18 days) the product, applied at first undiluted and later diluted with an equal volume of

benzene, was no more active (13 cancers and 6 papillomata from 86 mice in 292 days; the first tumour appeared on the 53rd day). (4) The residue from the action of 3 % AlCl_3 for 3 hours only, after distillation to 230° in the neck, gave no tumours. (5) The variability of commercial tetralin caused many difficulties; from some samples AlCl_3 produced little or no higher-boiling fraction. One of the most active preparations was made by Hieger from tetralin which he had converted into sulphonate and, after liberation, fractionated within very narrow limits (207.2° to 207.4°); this was then treated by Schroeter's method (2 % AlCl_3 for 9 hours at 65°) and then distilled at 15 mm. in nitrogen so as to give four fractions *a*, *b*, *c*, *d*, each of which was painted in solution in benzene on 50 mice. The results were as follows:

Fraction	Papillomata	Epitheliomata
<i>a.</i> Up to 205°	0	0
<i>b.</i> $205\text{--}260^\circ$	3	10
<i>c.</i> $260\text{--}320^\circ$	3	30
<i>d.</i> Residue	2	0

Evidently there was a considerable concentration of active substance in (*c*); the fluorescence spectrum of this fraction has been used extensively for comparison (see following paper). From this, and from some other experiments in which undistilled material has given negative results, it seems probable that the carcinogenic substance is produced by the heating in distillation, or at any rate is so increased in amount by this heating that it is able to produce tumours in mice (on the minimum concentration of such substances needed to produce cancer see Hieger [1929]). Hence the original object of these experiments, namely to produce carcinogenic substances at body temperature, may not have been attained. (6) Five similar series of experiments in which naphthalene, in place of tetralin, was acted on by AlCl_3 gave only 1 papilloma and 1 cancer from 130 mice.

The total yield of tumours produced by suitably prepared "Schroeter" has been 118 cancers and 30 papillomata from 496 mice. Further chemical and spectroscopic experiments are in progress upon the products from a very pure tetralin made by the staff of Technical Research Works, Ltd., to whom we are indebted for the great amount of care devoted to the preparation.

Schroeter [1924] found in the products of the action of AlCl_3 (2 %) upon tetralin (6–10 hours at $50\text{--}70^\circ$) benzene, octahydroanthracene (octhracene), octahydrophenanthrene (octanthrene), α -phenyl- δ -2-tetralylbutane, and 2 : 6'-ditetralyl (the last two identified in the oxidised forms as α -phenyl- δ -naphthylbutane and 2 : 2'-dinaphthyl respectively). By the further action of AlCl_3 upon octhracene, he obtained material yielding octanthrene, tetralin, and dodecahydrotriphenylene; from this he infers the production of some compounds (phenylene-bis-octhracenylobutane, tetralyloctanthrenylbutane) not isolated.

As a control upon the results obtained with "Schroeter," 30 mice were painted with untreated commercial tetralin; from these, one epithelioma was obtained on the 132nd day. 100 mice were then painted with tetralin which

had been purified by treatment with NaHSO_3 followed by rectification between 204° and 206° ; these yielded no tumours. Commercial tetralin may contain as much as 20 % of decalin; 10 mice which were painted with commercial decalin gave only a subcutaneous melanoma such as has occurred in various other series [cf. Twort and Twort, 1928], but no epithelial tumours. Dialin likewise gave negative results. The question whether the one cancer produced by commercial tetralin was due to an impurity, or to a special susceptibility of the mouse, must remain uncertain.

III. *Products of the action of aluminium chloride upon acetylene, xylene, and other substances.*

Hieger (unpublished observations) condensed acetylene by treatment with aluminium chloride at room temperature [Baud, 1900] and painted the non-volatile products, extracted in a Soxhlet with boiling benzene, upon mice. The difficulty of obtaining the material in quantity allowed of the painting of 30 mice only, among which 1 epithelioma and 1 cystic tumour of doubtful nature occurred. Thus acetylene yields carcinogenic products both (a) when passed through a red-hot tube, and (b) when acted upon by AlCl_3 with exposure to no temperature higher than the boiling point of benzene.

The products of the action of AlCl_3 upon various other substances (xylene, cholesterol, lanolin, sebaceous material from dermoids, human skin), of which some had given carcinogenic tars, were tested upon mice. All these gave negative results except the preparation from xylene, which yielded 1 papilloma and 1 cancer in 100 mice, and a papilloma upon the ear of a rabbit. High-boiling compounds, including apparently methylanthracenes, have been obtained by the action of AlCl_3 on xylene [Anschütz, 1886; Moore and Egloff, 1917].

COMPOUNDS RELATED TO NAPHTHALENE.

In view of the ease with which carcinogenic substances are obtained from tetralin, various compounds derived from, or closely related to, naphthalene were tested (Table II); all gave negative results. Most of the substances named in Tables II and III were applied in benzene. Many of the compounds of low molecular weight (*e.g.* phenylisocrotonic acid, cyclohexene) named in Tables II and III were taken, not with any expectation that they were themselves carcinogenic, but with the idea that they might serve as materials for synthesis in the skin of the mouse; this idea being suggested especially by the instance mentioned above, where an epithelioma arose in a mouse painted with tetralin only.

Table II.

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|-------------------------------|---------------------------------------|
| 1. Naphthalene. | 7. $\alpha\alpha$ -Dinaphthyl. |
| 2. Naphthoylbenzoic acid. | 8. α -Phenylnaphthalene. |
| 3. β -Naphthoic acid. | 9. β -2-Tetroylpropionic acid. |
| 4. Dinaphthylene dioxide. | 10. γ -2-Tetralylbutyric acid. |
| 5. β -Dinaphthyl ether. | 11. Phenylisocrotonic acid. |
| 6. $\beta\beta$ -Dinaphthyl. | |

Dinaphthylene dioxide was prepared because it has been stated to be an impurity which imparts the yellow colour to chrysene; the evidence for this seems quite insufficient, but the matter was too important to be left untested. In addition to the two dinaphthyls, crude reduction products (made by treatment with hydriodic acid and phosphorus) from both were tested in view of the presence of $\beta\beta$ -ditetralyl in "Schroeter." (8) was prepared by Hieger by the action of AlCl_3 upon $\text{C}_6\text{H}_5\text{Br}$ and C_{10}H_8 [Chattaway, 1893]. The distillates up to 342° containing the hydrocarbon were inactive, but the residue in the flask was strongly carcinogenic (6 cancers and 3 papillomata from 30 mice in 279 days); possibly a pyrogenous reaction had occurred producing compounds allied to those in "Schroeter." (9) gave no tumours, but the uncrystallisable oil from which it separates produced in 3 mice out of 20 cystic dilatations of hair follicles of doubtful nature; the acid is prepared [Krollpfeiffer and Schäfer, 1923] by the action of AlCl_3 upon tetralin and succinic anhydride, and hence may be accompanied by some compounds similar to those present in "Schroeter."

OTHER HYDROCARBONS AND QUINONES.

The following hydrocarbons, in addition to those named in the preceding tables, and quinones have been tested on mice.

Table III.

1. Cyclohexane.	8. Octracene.
2. Cyclohexene.	9. Octracenone.
3. Styrene.	10. 1 : 2-Benzanthracene.
4. Hydrophenanthrene.	11. 1 : 2-Benzanthraquinone.
5. Hydrochrysene.	12. 2 : 3-Tetrahydrobenzanthracene.
6. Hydrotene.	13. Perylene.
7. Dodecahydrotriphenylene.	

All the compounds named in Table III, except (10), gave negative results. (2) is extremely irritating to the skin, and should produce cancer if "chronic irritation" in general were an essential factor in carcinogenesis. The three reduced hydrocarbons (4), (5) and (6) were made by the methods of Schmidt and Mezger, and Liebermann and Spiegel, which are stated to give an octahydrophenanthrene $\text{C}_{14}\text{H}_{18}$, a hydrotene $\text{C}_{18}\text{H}_{20}$, and a perhydrochrysene $\text{C}_{18}\text{H}_{20}$; none of these was isolated in the pure state. (8), which is present in "Schroeter," and its quinone were made by the method of Krollpfeiffer and Schäfer [1923]. In addition to (7), the mixture of high-boiling compounds remaining when diphenyl is prepared from benzene and distilled off in the ordinary way, which is stated to contain benzerythrene and triphenylene, was tested with negative result. Diphenyl itself does not produce tumours (Bloch). Twort and Fulton report negative results at the time of writing with triphenylene and dodecahydrotriphenylene. In a mouse of the benzanthracene series (50 mice) a well-developed papilloma appeared, which however was only transitory; but in the light of the subsequent results with dibenzanthracenes

this was probably a significant observation. Perylene (for which, together with the phenanthrene-9-carboxylic acid required to make (10) in Table IV, we are indebted to the British Dyestuffs Corporation) is still under test.

The following hydrocarbons were synthesised by the methods of Clar and his fellow workers [1929] and of Fieser and Dietz [1929], and applied to mice, 10 to 20 mice being used for each substance. The selection of these compounds for special study was due to the spectroscopic work of Hieger, which is described in the following paper.

Table IV.

Hydrocarbon	Solution
1. 1 : 2 : 5 : 6-Dibenzanthracene.	Saturated, in benzene (less than 1 %)
2. 1 : 2 : 7 : 8-Dibenzanthracene	Saturated, in benzene (less than 2 %)
3. 1 : 2 : 3 : 4-Dibenzanthracene	Saturated, in benzene (less than 1 %)
4. Mixture of isomers of (3), probably naphtho-2' : 3' : 1 : 2-phenanthrene and naphtho-2' : 3' : 2 : 3-phenanthrene	Nearly saturated, in benzene
5. Naphtho-2' : 3' : 1 : 2-anthracene	1 % in benzene
6. 7 : 7'-Dimethyl-(naphtho-2' : 3' : 1 : 2-anthracene)	1 % in benzene
7. Anthraceno-2' : 1' : 1 : 2-anthracene	Saturated, in xylene
8. Anthraceno-1' : 2' : 1 : 2-anthracene	Saturated, in xylene
9. 3'-Methyl-1 : 2 : 5 : 6-dibenzanthracene	Saturated, in benzene (less than 0.5 %)
10. 1 : 2 : 3 : 4 : 5 : 6-Tribenzanthracene	0.3 % in benzene
11. 2 : 3 : 8 : 9-Di-[naphtho-1 : 2]-chrysene	Suspensions in tetralin and in oleic acid

Most of these were not highly purified, as this may require as many as twenty recrystallisations (Clar) involving considerable loss; but the preparations used should suffice to show whether the hydrocarbon in question is not carcinogenic. All the syntheses except that of (5), which involves a Grignard reaction, depend upon the action of AlCl_3 to produce the intermediate ketone. (10) and (11) were prepared by J. W. Cook. The compounds, except (7), (8) and (11), were dissolved in benzene for painting; the concentration was not in all cases the same but was dependent upon the amount available, and upon the solubility, of the compound. Sometimes the mother-liquor of the final recrystallisation was used, to economise material. (7) and (8) are very little soluble in lower boiling solvents, and were applied as the dilute saturated solutions in xylene; this is an undesirable solvent, as it produces considerable irritation and roughness, and occasional transient papillomata, in the skin of the mouse. (11), which is still more insoluble is applied in suspension in tetralin and in oleic acid. Some of these substances have at the time of writing (February, 1930) been applied to mice for a short time only.

10 mice were painted with (2). A mouse which died on the 203rd day showed changes suggesting the early stages of a papilloma. On the 169th day, one of the 6 mice surviving bore a papilloma; the animal was killed on the 223rd day, and the tumour was found to be clearly malignant, showing invasion of muscle over a wide area. Another mouse showed a papilloma on the 222nd day; the tumour grew rapidly, and when the mouse was killed a month later showed obvious invasion of muscle. At the date of writing (252nd day),

of the 2 surviving mice, one bears a papilloma, the other shows no tumour. Thus 2 cancers and 1 papilloma have been obtained in the series of 10 mice. 10 mice were painted with (1). On the 232nd day, 1 of the 3 mice surviving bore a well-developed papilloma which is growing at the present date; none of the others shows a tumour. (1) and (2) are almost identical in some physical properties, differing by about 1° in melting point.

The preparation of (2) used for painting should be fairly pure, being obtained from the mother liquor of crystals giving a very sharp melting point ($260-260.5^{\circ}$). All such results of course require confirmation with very pure material. The sample of (1) applied to mice was less pure than that of (2). These are the only instances in all the experiments described in this paper in which, as yet, cancers have been produced by a preparation of a fairly high degree of purity, for commercial tetralin, which caused one tumour, certainly could not be described in this way. The dibenzanthracenes can be only weak carcinogenic agents, for the incidence of tumours is low and the precancerous period long, but the result is an indication for further experiments, which are in progress¹. Apparently neither benzanthrane nor any of its derivatives have been found, and perhaps have not been sought for, in coal tar, and we are at present examining tar, and the products of the action of AlCl_3 upon tetralin, for such compounds. It is quite possible that there are, among the many compounds still undiscovered in coal tar, derivatives of benzanthrane or of chrysene which are far more powerfully carcinogenic than any known substances.

A number of new hydrocarbons (1-phenylanthracene, 2-phenylanthracene, 3-methyl-1:2-benzanthracene, 10-benzyl-1:2-benzanthracene, 6-phenyl-1:2-benzanthracene, 9:10-diphenyl-1:2-benzanthracene, acenaphthanthracene, 10-benzylacenaphthanthracene, 4'-methyl-1:2:7:8-dibenzanthracene, 2:3-benzo-8:9-[1':2'-naphtho]-chrysene) have been synthesised by J. W. Cook in this laboratory, by methods which he will describe in the *Journal of the Chemical Society*; all these compounds are being tested on mice.

SUMMARY.

1. Carcinogenic products have been obtained by the action of aluminium chloride upon (a) acetylene, (b) xylene, (c) naphthalene, (d) naphthalene and bromobenzene, and (e) tetrahydronaphthalene. Of these (d) and (e), under the conditions described, give the most active materials.

2. Two cancers have been obtained in a series of 10 mice painted with a solution of a fairly pure specimen of 1:2:7:8-dibenzanthracene.

¹ (Note added at correction of proof.) Two more cancers have been obtained from the mice of the 1:2:7:8-dibenzanthracene series; thus the last four of the 10 mice painted with this material have all given cancers. The experiments will be repeated with a sample of the hydrocarbon freed completely from yellow colouring matter. The last four of the 10 mice painted with impure 3'-methyl-1:2:5:6-dibenzanthracene have developed papillomata.

I wish to express my indebtedness to the British Empire Cancer Campaign, whose grants to I. Hieger have enabled him to develop methods which have been essential to the experiments described here. Dr E. de Barry Barnett has most kindly given many valuable specimens of pure hydrocarbons for use in this laboratory. It is a pleasure to record the very large part that has been played throughout these investigations by the work of my assistant F. Goulden.

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